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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/779,560	02/09/2001	Marianne Harboe	58982.000002	6162

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EXAMINER	
STEADMAN, DAVID J	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 02/09/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/779,560	HARBOE, MARIANNE	
	Examiner	Art Unit	
	David J Steadman	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 December 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6,9-18,29-31,35,36,39 and 42* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,9-18,29-31,35,36,39 and 42* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Status of the Application

[1] A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 12, 2003, has been entered.

[2] Claims 1-6, 9-18, 29-31, 35-36, 39, and 42 are pending in the application.

[3] Applicants' amendment to the claims, filed July 25, 2003, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims in the instant application.

[4] Applicants' arguments, filed June 19, 2003 and July 25, 2003, have been fully considered and are deemed to be persuasive to overcome some of the rejections and/or objections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

[5] The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Claim Objections

[6] Claim 12 is objected to because of the following informalities: the term "*Rhizomuor*" is misspelled and should be replaced with, for example, "*Rhizomucor*". Appropriate correction is required.

[7] Claim 15 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 14 (from which claim 15 depends) recites a pH between 1.7 and 1.75. Claim 15 does not further limit claim 14 as claim 15 recites a pH between 1.75 and 1.8. It is suggested that, for example, applicants amend claim 15 to depend from claim 13.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

[8] Claim(s) 1-6, 9-18, 29-31, 35-36, and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laustsen et al. (US Patent 6,080,564; originally cited in the Office action mailed April 09, 2002) in view of Larson et al. (WO 95/29999; cited in the IDS filed April 16, 2001), Heinsohn et al. (US Patent 5,215,908; originally cited in the Office action mailed April 09, 2002), Ward et al. (*Biotechnol* 8:435-440; cited in the IDS

filed April 16, 2001), and Thomas et al. (*J Agric Food Chem* 32:825-828). The claims are drawn to a method of providing a chymosin preparation having a content of glucoamylase activity at such a level that it does not restrict the applicability of chymosin for its intended use, the method comprising the steps of: (i) providing a medium of 2.0 or higher comprising chymosin and glucoamylase and (ii) subjecting the medium to a pH between about 1.7 and about 1.9 for a period of time sufficient to at least partially inactivate the glucoamylase, while maintaining at least partial activity of the chymosin.

A method of inactivating an undesirable enzyme, while maintaining a desired enzyme activity by decreasing pH of a medium comprising the undesired and desired enzymes was well known in the art at the time of the invention as represented by Laustsen et al. Laustsen et al. teach a method of obtaining a desirable enzyme with inactivated undesired enzyme activities by treatment with low pH (column 1, top). Laustsen et al. teach that the undesirable enzyme may be obtained from plant, animal, or microbial sources (column 2, bottom) and that the desirable enzymes may be obtained from plant, animal, or microbial sources (column 3, top). Laustsen et al. teach a method of determining optimal conditions (pH, time, and temperature) for obtaining a desired polypeptide with inactivated undesired enzymatic activities by determining the pH optimum of the desired polypeptide (column 4, top). Laustsen et al. teach it is advantageous to hold the pH as acidic as possible for a desired polypeptide with an acidic pH optimum (column 4, top). Laustsen et al. teach that the pH can be adjusted using an inorganic or organic acid (column 4, bottom). Laustsen et al. teach the time required to inactivate the undesired enzymes can typically range from 20 seconds to up

to 2 weeks (column 4, middle). Laustsen et al. teach the undesired enzyme should be inactivated to a level of at least 1-15% of the original undesired enzyme activity (column 4, bottom). Laustsen et al. teaches the desired polypeptide should maintain at least 50% of the original desired polypeptide activity (column 4, bottom). Laustsen et al. provide examples of the disclosed method by treating an *Aspergillus* medium with low pH, thereby significantly reducing undesired amylase activity at a pH of 3.5 (Example 4), while maintaining desired enzyme activity. Laustsen et al. do not specifically teach chymosin as a desired polypeptide, glucoamylase as an undesired polypeptide, and the use of a pH of less than 2.0 to reduce glucoamylase enzyme activity.

The ability of chymosin to maintain enzymatic activity at a pH at or below 2.0 was well known in the art at the time of the invention. For example Larson et al. teach treatment of a crude extract comprising chymosin with an organic or inorganic acid at a pH of as low as 0.5 (page 10, middle to bottom), which results in activation of the chymosin. Also, Heinsohn teaches treating an *Aspergillus* culture growth medium comprising chymosin with a pH of about 2 in order to stop fermentation and cell growth of the cultured cells as a first step in the purification of the chymosin (column 2, bottom).

The recombinant expression of chymosin using various hosts was well known in the art at the time of the invention. For example, Ward et al. teach *Escherichia coli*, *Saccharomyces cerevisiae*, and *Yarrowia lipolytica* have been used successfully as hosts for expression of prochymosin cDNA (page 435, right column, middle). Ward et al. teach that expression of prochymosin as a fusion in these host cells yielded increased intracellular or extracellular expression (page 435, right column). Ward et al. teach a

vector encoding a glucoamylase-bovine prochymosin B fusion protein for expression in *Aspergillus niger* var. *awamori* (page 435, abstract). Ward et al. teach that following expression and secretion of the fusion protein using *Aspergillus niger* var. *awamori* as a host, treatment at pH 2.0 released the glucoamylase from the chymosin (page 435, middle). Ward et al. teach a significant increase in *Aspergillus niger* var. *awamori* secretion of chymosin when fused to glucoamylase (page 437, Table 2).

The presence of glucoamylase enzymatic activity as a contaminant in preparations of chymosin was well known in the art at the time of the invention as evidenced by Thomas et al. (see, *e.g.,* abstract and page 825, right column).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Laustsen et al., Larson et al., Heinsohn et al., Ward et al., and Thomas et al. to express chymosin in a host, *e.g., E. coli, S. cerevisiae,* or *Aspergillus niger* var. *awamori* and treat the culture medium and/or extract at a pH as low as 0.5. One would have been motivated to express chymosin in a host, *e.g., E. coli, S. cerevisiae,* or *Aspergillus niger* var. *awamori* and treat the culture medium and/or extract at a pH as low as 0.5 in order to reduce contaminating enzyme activities – specifically glucoamylase activity, activate the expressed chymosin, and to stop fermentation and cell growth of the cultured cells, all in a single step. One would have a reasonable expectation of success for expressing chymosin in a host, *e.g., E. coli, S. cerevisiae,* or *Aspergillus niger* var. *awamori* and treating the culture medium and/or extract at a pH as low as 0.5 because of the teachings of Laustsen et al., Larson et al., Heinsohn et al., and Ward et al. as described above. Therefore, claims 1-6, 9-18, 29-31,

35-36, and 42, drawn to the method described above would have been obvious to one of ordinary skill in the art.

[9] Claim(s) 39 is rejected under 35 U.S.C. 103(a) as being unpatentable over Laustsen et al. in view of Larson et al., Heinsohn et al., Ward et al., and Thomas as applied to claims 1-6, 9-18, 29-31, 35-36, and 42 above and further in view of Kappeler et al. (US Published Patent Application 2002/0164696 A1). The claim limits the chymosin of the method of claim 1 to being a *Camelus dromedarius* chymosin.

The teachings of Laustsen et al., Larson et al., Heinsohn et al., Ward et al., and Thomas et al. are described above. None of the references of Laustsen et al., Larson et al., Heinsohn et al., Ward et al., or Thomas et al. teaches a chymosin from *Camelus dromedarius.*

Kappeler et al. teach the isolation of a nucleic acid encoding *Camelus dromedarius* chymosin and recombinant expression thereof using *Aspergillus niger* var. *awamori* as an expression host (see Examples 1-3). Kappeler et al. teach that a comparison of the clotting activities of camel and bovine chymosins reveals that camel chymosin has 170-180% of the clotting activity of bovine chymosin with less non-specific proteolytic activity than bovine chymosin (see Example 5).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Laustsen et al., Larson et al., Heinsohn et al., Ward et al., Thomas et al., and Kappeler et al. to practice the method of Ward et al. for expressing a glucoamylase-camel chymosin fusion protein and treating the resulting culture medium at a pH as low as 0.5. One would have been motivated to practice the method of Ward

et al. for expressing a glucoamylase-camel chymosin fusion protein and treating the resulting culture medium at a pH as low as 0.5 in order to obtain camel chymosin for use in milk clotting/cheese production and to reduce contaminating enzyme activities – specifically glucoamylase activity, activate the expressed chymosin, and to stop fermentation and cell growth of the cultured cells, all in a single step. One would have a reasonable expectation of success for practicing the method of Ward et al. for expressing a glucoamylase-camel chymosin fusion protein and treating the resulting culture medium at a pH as low as 0.5 because of the teachings of Laustsen et al., Larson et al., Heinsohn et al., Ward et al., and Kappeler et al. as described above. Therefore, claim 39, drawn to the method described above would have been obvious to one of ordinary skill in the art.

[10] It is noted that the arguments presented in the amendments filed June 19, 2003 and July 25, 2003 appear to be identical and therefore have not been separately addressed. Applicants argue the combined references do not teach or suggest the claimed method. Applicants argue that given the purposes of Ward et al. and Heinsohn et al., one would not be motivated to combine the references to achieve the claimed method, particularly given the limitations of chymosin as the polypeptide and glucoamylase as the undesired polypeptide. Applicants' argument is not found persuasive.

Irrespective of the purposes of the individual prior art references, they nonetheless combine to provide motivation to practice the claimed invention. Contrary to applicant's assertion, there is clear motivation to combine the cited references, which

is to reduce contaminating enzyme activities, *e.g.,* glucoamylase activity, activate the expressed chymosin, and to stop fermentation and cell growth of the cultured cells, all in a single step as described above. This motivation is specific to the limitations of chymosin as a protein that maintains partial activity and glucoamylase as the undesired protein.

Conclusion

[11] Status of the claims:

- Claims 1-6, 9-18, 29-31, 35-36, 39, and 42 are pending.
- Claims 1-6, 9-18, 29-31, 35-36, 39, and 42 are rejected.
- No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (571) 272-0942. The Examiner can normally be reached Monday-Friday from 7:30 am to 4:00 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (571) 272-0928. The FAX number for submission of official papers to Group 1600 is (703) 308-4242. Draft or informal FAX communications should be directed to (571) 273-0942. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.
Patent Examiner
Art Unit 1652

02-03-04

DAVID STEADMAN
PATENT EXAMINER